U.S.P 6,835,829
6,284,888

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07D 501/18, 501/22, 501/12

A1

(11) International Publication Number: **WO 97/07121**

(43) International Publication Date: 27 February 1997 (27.02.97)

(21) International Application Number: PCT/EP96/03582

(22) International Filing Date: 13 August 1996 (13.08.96)

(30) Priority Data:
A 1369/95 14 August 1995 (14.08.95) AT

(71) Applicant *(for all designated States except US)*: BIOCHEMIE GESELLSCHAFT MBH [AT/AT]; A-6250 Kundl (AT).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: LUDESCHER, Johannes [AT/AT]; Kleinsoell 101, A-6252 Breitenbach (AT). VEIT, Werner [AT/AT]; Kaiserjägerstrasse 27, A-6330 Kufstein (AT).

(74) Agents: WYMANN, Gerard et al.; Sandoz Technology Ltd., Patents & Trademarks Div., CH-4002 Basle (CH).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: PURIFICATION PROCESS

(57) Abstract

Processes for the depletion of 7-ADCA in mixtures of vinyl-ACA with 7-ADCA via novel salts of vinyl-ACA or via chromatography.

FOR THE PURPOSES OF INFORMATION ONLY

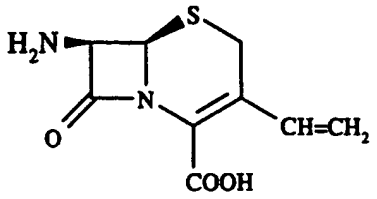
Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgystan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SG	Singapore
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LR	Liberia	SZ	Swaziland
CS	Czechoslovakia	LT	Lithuania	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	LV	Latvia	TJ	Tajikistan
DK	Denmark	MC	Monaco	TT	Trinidad and Tobago
EE	Estonia	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	UG	Uganda
FI	Finland	ML	Mali	US	United States of America
FR	France	MN	Mongolia	UZ	Uzbekistan
GA	Gabon	MR	Mauritania	VN	Viet Nam

Purification Process

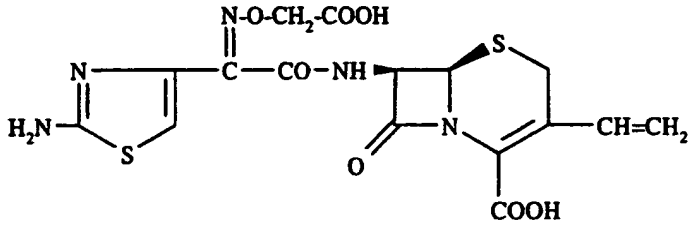
The present invention relates to a process for the purification of vinyl-ACA in a mixture of vinyl-ACA and 7-ADCA, by depletion of 7-ADCA in a mixture of vinyl-ACA and 7-ADCA.

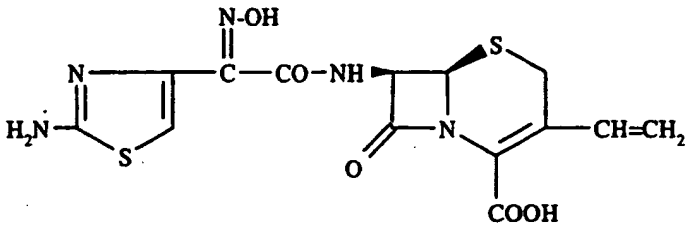
Vinyl-ACA of formula



I

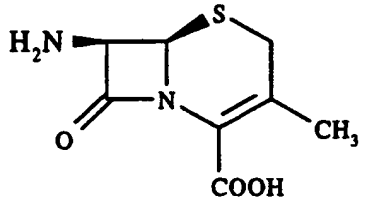
may be used as intermediate in the production of highly active, oral antibiotics, *e.g.* cefixime and cefdinir of formulae





Vinyl-ACA may be produced, for example, by Wittig reaction of a corresponding cephalosporin-3-ylide, which may have the amine group and the carboxylic acid attached to the ring system protected, with formaldehyde (see *e.g.* Journal of Antibiotics, Vol. 38, No. 12, 1739 ff; or EP-0 503 453; or EP-0 597 429). We have found that such vinyl-ACA

may be contaminated, *e.g.* by 7-ADCA. This is consistent with the fact that phosphine alkylenes (ylides) or quaternary phosphonium compounds can hydrolyse to form the corresponding alkane and phosphine oxide (see *e.g.* Houben Weyl, Methoden der organischen Chemic, Phosphorverbindungen I, volume 12/1, especially pages 108 and 119). We found accordingly, when producing a compound of formula I *via* a Wittig reaction, as a by-product 7-ADCA of formula



II

or a protected derivative thereof may be formed. Furthermore, if 7-ACA is produced *via* fermentative production of cephalosporin C and subsequent conversion to 7-ACA, the thus formed 7-ACA may contain 7-ADCA, because the 7-ADCA-analogous cephalosporin is formed in the course of fermentation, or is not wholly metabolised to cephalosporin C. Thus, 7-ACA, used for example as a starting material for 7-ACA, may often have an undesired 7-ADCA content, for example of more than 1%.

In the production of active cephalosporins, *e.g.* cefixime and cefdinir, wherein an intermediate of formula I may be used, the 7-ADCA content in vinyl-ACA of formula I should be as low as possible, because upon appropriate further substitution of a compound of formula I, 7-ADCA could react in the same way as vinyl-ACA which would result in contamination of the desired active vinyl-ACA compounds, e.g. cefixime or cefdinir, by analoguously substituted 7-ADCA-compounds, which are difficult to separate.

According to the present invention, vinyl-ACA in a mixture of vinyl-ACA and 7-ADCA can be purified in an economical manner by depletion of 7-ADCA to, *e.g.* less than 0.1% to 0.8%, such as less than 0.1% to 0.6%, for example 0.3% to 0.8%. This is remarkable, because 7-ADCA and vinyl-ACA are chemically very similar compounds.

In one aspect the present invention provides a process for the depletion of 7-ADCA of

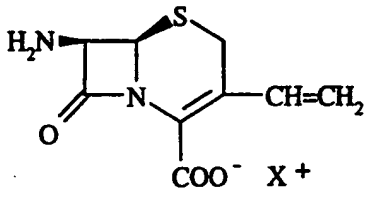
formula II in a mixture of 7-ADCA and vinyl-ACA of formula I, preferably by a process, wherein

a) a mixture of a salt of a compound of formula I and a compound of formula II is subjected to crystallization, the crystallised salt is isolated and converted into a compound of formula I, containing less compound of formula II than the mixture of a compound of formula I and formula II, or

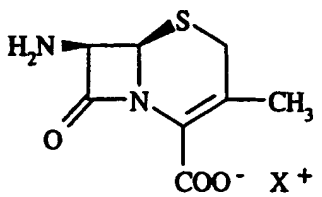
b) a mixture of a compound of formula I and a compound of formula II is subjected to chromatography.

The salt includes for example a cationic salt of the carboxylic acid group and an amine salt of the carboxylic acid group in a compound of formulae I and II.

In a further aspect the present invention provides a process as described above, wherein a mixture of a salt of a compound of formula I and a compound of formula II is a mixture of compounds of formulae

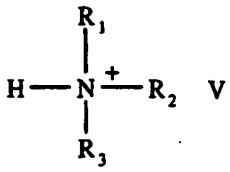


III



IV

wherein X^+ denotes a cation, or a compound of formula



V

wherein R_1, R_2 and R_3 are the same or different and independently of one another denote hydrogen, alkyl, aryl, aralkyl, or cycloalkyl; or

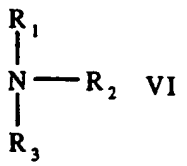
R_1 and R_2 together with the nitrogen atom form a heterocycle and R_3 is as defined above.

The cation includes a cation of the alkali series, for example Li^+, K^+, Na^+.

Preferably R_1 denotes hydrogen and R_2 and R_3 independently from one another denote alkyl or aralkyl. R_1 and R_2 together with the nitrogen atom may denote a heterocycle, preferably a 5- or 6 membered heterocycle, having for example 1 to 3 heteroatoms.

If not otherwise defined herein, any carbon containing radical contains up to 10 carbon atoms. Alkyl includes straight chain or branched C_{1-22}alkyl, preferably C_{1-12}alkyl, such as C_{1-8}alkyl. Aryl includes unsubstituted aryl or substituted aryl, preferably phenyl or, mono- or polysubstituted phenyl. Aralkyl includes unsubstituted aralkyl, or substituted aralkyl, for example benzyl. Cycloalkyl includes C_{3-8}cycloalkyl, such as C_{3-6}cycloalkyl. A heterocycle includes unsubstituted heterocycle or substituted heterocycle, for example 5- or 6-membered heterocycle. A heterocycle may contain one or several heteroatoms, for example N, S, O. Substituents of any aryl group, aralkyl group and of any heterocycle include substitutents which are inert under the corresponding reaction conditions, for example alkyl, aryl, alkoxy, aryloxy, halogen, nitro, optionally protected amine groups, optionally protected hydroxy.

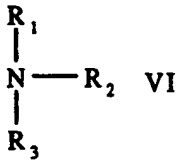
Process variant a) may be carried out as follows:
A salt of a mixture of a compound of formula I and of formula II may be produced, for example, by adding a salt forming agent to a mixture of a 7-ADCA and vinyl-ACA in a solvent. A salt forming agent includes, for example, a base. A base includes, *e.g.* an inorganic base, *e.g.* a hydroxide, for example an alkali hydroxide; and a salt having a cation source; such as an inorganic salt, for example an alkali salt, such as a carbonate, hydrogencarbonate; and an organic salt, for example the salt of a carboxylic acid, for example an alkali salt, of, for example acetic acid or 2-ethylhexanoic acid; and an organic base, for example a nitrogen base, for example ammonia or an amine, for example an amine of formula



VI

wherein R_1, R_2 and R_3 are as defined above.

In a further aspect the present invention provides a process as described above, wherein a mixture of a salt of a compound of formula I and a compound of formula II is produced by addition of a salt forming agent to a mixture of a compound of formula I as defined in claim 1 and a compound of formula II as defined in claim 1 in a solvent, preferably a process, wherein the salt forming agent is an inorganic base, an inorganic salt, an organic salt or a nitrogen base; preferably, the inorganic base is a hydroxide; the inorganic salt is an inorganic alkali salt; the organic salt is an alkali salt of a carboxylic acid; and the nitrogen base is a compound of formula



VI

wherein R_1, R_2 and R_3 are as defined above.

A solvent includes an aprotic solvent and a protic solvent, for example an amide, such as N,N-dimethylform-amide, a ketone, such as acetone; an alcohol, such as methanol, ethanol or one of the isomeric propanols or butanols, for example isopropanol; a nitrile such as acetonitrile; ethers or chlorinated hydrocarbons; water; and mixtures of solvents.

In one aspect a mixture of vinyl-ACA and 7-ADCA may be dissolved in water or in an aqueous organic solvent, for example a mixture of water and a ketone; and a mixture of water and an alcohol, such as ethanol or isopropanol; in the presence of a salt forming agent. The pH may be appropriately adjusted, for example by addition of a base, having, for example, a Li, Na or K-source, such as an acetate; particularly in case that an organic base, such as a nitrogen base is used as salt forming agent. An anti-solvent, for example a nitrile, such as acetonitrile; an alcohol such as methanol, ethanol or one of the isomeric propanols or butanols; an ether, such as diethyl ether, tetrahydrofuran or tert.butylmethyl ether; a ketone, such as acetone; or an ester, such as ethyl acetate or acetic acid isopropyl ester; or mixtures of anti-solvent; may be added. The salt of vinyl-ACA, or a mixture of a salt of vinyl-ACA and 7-ADCA, wherein the 7-ADCA content is less than in the mixture

used for salt production, may crystallize.

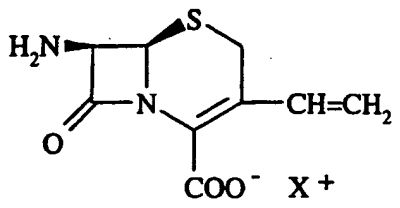
In another aspect a mixture of vinyl-ACA and 7-ADCA may be suspended in a practically water-free organic solvent, such as an amide, a ketone; an alcohol; a nitrile; an ether; a chlorinated hydrocarbon; and mixtures of water-free organic solvent. Preferred solvent include a mixture of methanol with a ketone or a higher alcohol. A nitrogen base, for example of formula VI, and an anti-solvent as defined above may be added.

Surprisingly the undesired salt of 7-ADCA may be better soluble than the desired salt of vinyl-ACA and a salt of vinyl-ACA or a mixture of a salt of vinyl-ACA and 7-ADCA in which the content of 7-ADCA is less than in the mixture used for salt production is precipitated. By isolation of the precipitate which may be carried out as conventional, separation of a compound of formula III and of a compound of formula IV may be effected.

If required, process variant a) may be repeated with a product obtained according to process variant a) resulting in further depletion of the undesired compound of formula II. A mixture of a salt of vinyl-ACA and 7-ADCA obtained may be resuspended in the solvent system in which crystallisation had been carried out, and the solubility product may be adjusted, for example by addition of solvent or anti-solvent as appropriate, with the effect of further depletion of 7-ADCA.

A compound of formula III in crystalline form is new.

In another aspect the present invention provides a compound of formula



III

wherein X^+ is as defined above, in crystalline form.

In another aspect the present invention provides the

- dicyclohexylammonium salt of 7-amino-3-vinyl-cephalosporanic acid in crystalline form;
- tert.octylammonium salt of 7-amino-3-vinyl-cephalosporanic acid in crystalline form;
- N-benzyl-tert.butylammonium salt of 7-amino-3-vinyl-cephalosporanic acid in crystalline form;
- 2-ethyl-1-hexylammonium salt of 7-amino-3-vinyl-cephalosporanic acid in crystalline form;
- potassium salt of 7-amino-3-vinyl-cephalosporanic acid in crystalline form.

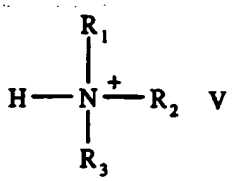
The salt of vinyl-ACA or of the mixture of vinyl-ACA and 7-ADCA may be isolated and converted into the free compound of formula I and formula II as conventional, for example by treatment with an acid, such as an inorganic acid, such as hydrochloric acid, sulphuric acid, preferably sulphuric acid; or an organic acid such as an organic carboxylic acid.

Process variant b) according to the present invention may be carried out as follows:
Chromatography preferably may be adsorption chromatography.
A mixture of vinyl-ACA and 7-ADCA may be dissolved, for example in water, for example in the presence of a base, e.g. ammonia. This solution is subjected to chromatography using an adsorbent. An adsorbent includes activated charcoal, e.g. Norit CG-1 or Cecarbon GAC 40; or an adsorber resin, such as styrene-divinylbenzene copolymerisates, for example Dianion HP 20 (CAS No. 55353-13-4), Dianion HP 21 (Cas No. 92529-04-9) or Dianion SP 207 (CAS No. 98225-81-1) from Mitsubishi Kasei Corporation; Amberlite XAD 1180 (CAS No. 97396-56-0), Amberlite XAD 1600 (CAS No. 153796-66-8) or Amberlite XAD 16 (CAS No. 102419-63-8) from Rohm and Haas or Amberchrom CG 161 (CAS No. 131688-63-6) from TosoHaas; preferably CG 161 and XAD-1600. Elution may be carried out with water. The compound which elutes earliest may be in general 7-ADCA. Thus, fractions of the compound of formula II, mixtures of the compound of formula I and II and the pure compound of formula I may be obtained. Isolation of a compound of formula I may be carried out by adjustment of the pH of a

fraction containing a compound of formula I obtained by the present invention to around the isoelectric point of a compound of formula I, for example as conventional, *e.g.* by addition of an acid, such as an inorganic acid, for example hydrochloric acid. The compound of formula I may crystallise.

Process variant b) is very simple to carry out. Elution may be effected with a purely aqueous medium, no organic solvent is to be used. The equipment required is simple. We have found that there is no need for elution by means of a gradient, nor step-wise elution, nor pH changes in the course of chromatographic purification.
Process variant a) can be combined with process variant b) for still more effective depletion of 7-ADCA in a mixture of vinyl-ACA and 7-ADCA.

In another aspect the present invention provides the use of a compound of formula

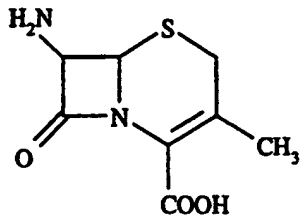


V

wherein X^+ is as defined above, or the use of a process for the depletion of 7-ADCA of formula II in a mixture of 7-ADCA and vinyl-ACA of formula I, in the production of highly active cephalosporins, for example cefixime and cefdinir.

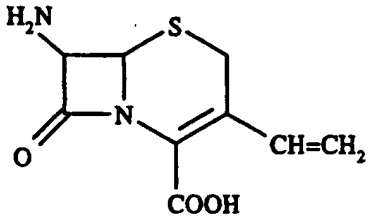
Processes a) and b) of the present invention represent very economical methods of separating 7-ADCA from mixtures of vinyl-ACA with 7-ADCA, which are very simple to carry out and which are suitable for use on industrial scale.

In another aspect the present invention provides a depletion process of 7-ADCA of formula



II

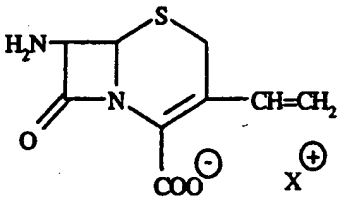
in mixtures of vinyl-ACA of formula



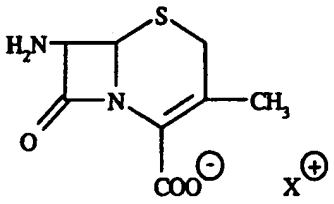
I

with 7-ADCA, characterised in that

a) a mixture of vinyl-ACA and 7-ADCA is converted into salts of formula

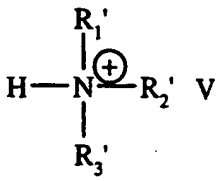


III



IV

wherein $X^{\oplus}$ is $Li^{\oplus}$, $Na^{\oplus}$, $K^{\oplus}$ or a cation of formula

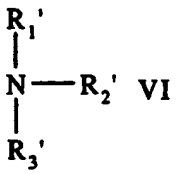


V

wherein R_1', R_2' and R_3' are the same or different and independently of one

another denote hydrogen, (C_{1-8})alkyl, optionally substituted benzyl or phenyl or (C_{4-8})cycloalkyl, or R_1' and R_2' together with the nitrogen form a 5- or 6-membered heterocycle which optionally contains a further one or two hetero atoms, and R_3' is as defined above,

by reaction of the mixture of vinyl-ACA and 7-ADCA with a lithium, sodium or potassium base or with an amine of formula



VI

wherein R_1', R_2' and R_3' are as defined above, whereby

α) the reaction is carried out in a solvent or solvent mixture in which the salts of formulae III and IV have different solubilities, or

β) the salts of the compounds of formulae III and IV are suspended in a solvent or solvent mixture, and the solubility product is adjusted,

and after isolating the compound of formula III, this is converted using an acid into the compound of formula I having no content or a reduced content of 7-ADCA, or

b) a solution of a mixture of vinyl-ACA with 7-ADCA is chromatographed.

In the following examples all temperatures are given in degrees celsius.

The following abbrevations are used:

Vinyl-ACA:	Compound of formula I
7-ADCA:	Compound of formula II
GC:	Gas chromatography
KF:	Karl Fischer
HPLC:	High performance liquid chromatography

Example 1: Purification of viny-ACA *via* the dicyclohexylammonium salt

1a) Dicyclohexylammonium salt of vinyl-ACA

12.7 g of vinyl-ACA, containing 1.1% 7-ADCA (HPLC), are suspended in a mixture of 56.5 ml of acetone and 3.75 ml of water. 12 ml of dicyclohexylamine are added in one portion. The mixture is stirred for ca. 5 minutes. A solution is obtained, stirring is stopped, and the solution is left to stand for ca. 15 minutes. Crystallisation starts. The crystal mass is agitated. 115 ml of acetone are added dropwise within ca. 30 minutes. The reaction mixture is stirred for 2 hours at -10°, the crystalline precipitate is isolated, washed with acetone, and dried.

19.3 g of the dicyclohexylammonium salt of vinyl-ACA are obtained in crystalline form.

Analysis:

Vinyl-ACA	48.4 %	(HPLC)
7-ADCA	0.3 %	(HPLC)
Dicyclohexylamine	37.7 %	(GC)
Acetone	10.2 %	(GC)
H_2O	3.7 %	(KF)

^{1}H-NMR (D_2O, trimethylsilylpropionic acid sodium salt-d_4): 6.71 (dd, J=11 and 18, C=CH); 5.40 (d, J=18, C=CH_2); 5.22 (d, J=11, C=CH_2); 5.07 (d, J=5, H_7); 4.75 (d, J=5, H_6); 3.70 (d, J=17, H_2); 3.56 (d, J=17, H_2); 3.12-3.70 (m); 2.00 (br); 1.79 (br); 1.65 (m); 1.00-1.34 (m).

1b) Dicyclohexylammonium salt of vinyl-ACA

2.26 g of vinyl-ACA, containing 0.5% of 7-ADCA, are suspended in a mixture of 11.3 ml of acetone and 1 ml of water. 2.4 ml of dicyclohexylamine are added under stirring and a solution is obtained. Crystallisation starts, stirring is stopped and the resultant suspension is left to stand for 10 minutes. The crystal mass is agitated and 22.6 ml of acetone are added dropwise within ca. 10 minutes. The suspension is stirred for 30 minutes at room temperature, the precipitate is filtered off, washed with acetone, and dried.

3.0 g of the dicyclohexylammonium salt of vinyl-ACA are obtained in crystalline form.

Analysis:

Vinyl-ACA	52.4 %	(HPLC)
7-ADCA	0.2 %	(HPLC)

Dicyclohexylamine	39.6 %	(GC)
Acetone	3.6 %	(GC)
H_2O	0.7 %	(KF)

1c) Vinyl-ACA *via* dicyclohexylammonium salt of vinyl-ACA

8.15 g of the dicyclohexylammonium salt of vinyl-ACA, obtained as described in Example 1a), are dissolved in 160 ml of water at room temperature. The solution is stirred for 10 minutes with 0.8 g of activated charcoal. The charcoal is filtered off and the filter washed with 25 ml of water. The filtrate is adjusted to pH 3.4 with 10 N H_2SO_4 at room temperature within ca. 15 minutes. Precipitation occurs. The suspension is stirred for 1 hour whilst cooling with ice, the precipitate is isolated through a suction filter, washed with 3 x 10 ml of water and 3 x 10 ml of acetone and dried.

3.9 g of vinyl-ACA, containing 0.5% 7-ADCA, are obtained.

Example 2: Purification of vinyl-ACA *via* the tert.octylammonium salt of vinyl-ACA

2a) Tert.octylammonium salt of vinyl-ACA

12.7 g of vinyl-ACA, containing 1.1% 7-ADCA, are suspended in a mixture of 56.5 ml of acetone and 56.5 ml of water. 10 ml of tert.octylamine are added in one portion. A solution is obtained within ca. 5 minutes. 226 ml of acetone are added. Stirring is stopped. Crystallization occurs. After ca. 15 minutes, the crystal mass is agitated, and 400 ml of acetone are added dropwise within ca. 30 minutes. The suspension is stirred for 2 hours whilst cooling with ice, the precipitate is isolated, washed with 50 ml of acetone and dried.

18.9 g of the tert.octylammonium salt of vinyl-ACA are obtained in crystalline form.

Analysis:

Vinyl-ACA	56.8%	(HPLC)
7-ADCA	0.5%	(HPLC)
Tert.octylamine	30.6%	(GC)
Acetone	8.4%	(GC)
H_2O	3.3%	(GC)

^{1}H-NMR (methanol-d_4, D_2O): 6.91 (dd, J=11 and 18, C=CH); 5.31 (d, J=18, C=CH_2); 5.11 (d, J=11, C=CH_2); 5.01 (d, J=5, br, H_7); 4.67 (d, J=5, br, H_6); 3.65 (d, J=17, H_2); 3.52 (d,

J=17, H_2); 1.66 (s, CH_2); 1.43 (s, CH_3); 1.05 (s, CH_3).

2b) Tert.octylammonium salt of vinyl-ACA

2.26 g of vinyl-ACA, containing 0.8% of 7-ADCA, are suspended in a mixture of 11.3 ml of acetone and 11.3 ml of water. 2 ml of tert.octylamine are added and a solution is obtained. 22.6 ml of acetone are added in one portion, and the mixture is left to stand for 10 minutes. Crystallisation occurs. The crystal mass is agitated and mixed with a further 22.6 ml of acetone. The suspension is stirred for ca. further 30 minutes at room temperature, the precipitate is isolated and washed twice, each time with 10 ml of acetone. The product is dried.

3.0 g of the tert.octylammonium salt of vinyl-ACA are obtained in crystalline form.

Analysis:

Vinyl-ACA	57.1%	(HPLC)
7-ADCA	0.3 %	(HPLC)
Tert.octylamin	31.0 %	(GC)
Acetone	5.8 %	(GC)
H_2O	2.1 %	(KF)

2c) Vinyl-ACA *via* the tert.octylammonium salt of vinyl-ACA

7.11 g of the tert.octylammonium salt of vinyl-ACA, obtained as described in Example 2a), are dissolved in 280 ml of water at 35° and stirred for 10 minutes with 0.7 g of activated charcoal. The charcoal is filtered off and the filter washed with 25 ml of water. The filtrate is cooled to 5° with ice water, and a pH of 3.4 is adjusted with 10 N H_2SO_4 within 15 minutes. The resultant suspension is stirred for ca. 30 minutes whilst cooling with ice, the precipitate is isolated, washed with 3 x 10 ml of water and 3 x 10 ml of acetone and dried.

4.0 g of vinyl-ACA, containing 0.8% of 7-ADCA, are obtained.

Example 3: Purification of vinyl-ACA *via* the potassium salt of vinyl-ACA

3a) Potassium salt of vinyl-ACA

10 g vinyl-ACA, containing 1.0% of 7-ADCA (HPLC), are suspended in a mixture of 40 ml of 95% ethanol and 5 ml of water, and the suspension is cooled with ice water. 8.0

ml of triethylamine are added dropwise whilst stirring, the resultant solution is filtered and the filtrate is mixed with a solution of 10 g of potassium acetate in 15 ml of ethanol. Crystallisation occurs. The crystal mass is agitated, and stirred for 15 minutes whilst cooling with ice. The precipitate is isolated by filtration, washed with ethanol and dried. 6.7 g of the potassium salt of vinyl-ACA are obtained in crystalline form.

Analysis

Vinyl-ACA	78.7 %	(HPLC)
7-ADCA	0.4 %	(HPLC)
EtOH	0.7 %	(GC)
H_2O	1.8 %	(KF)

3b) Potassium salt of vinyl-ACA

3 g of the potassium salt of vinyl-ACA, containing 0.4% of the potassium salt of 7-ADCA, obtained according to example 3a), are dissolved in 50 ml of water. 0.3 g of activated charcoal are added and the mixture is stirred for ca. 10 minutes at room temperature. The charcoal is filtered off, and the filter washed with 10 ml of water. The filtrate is cooled to 5°. The pH is adjusted to 3.4 with 10 N H_2SO_4 within ca. 15 minutes. After stirring for 1 hour whilst cooling with ice, the precipitate is isolated, washed 3 x with 10 ml of water and 3 x with 10 ml of acetone, and dried.

2.38 g of the potassium salt of vinyl-ACA, containing 0.3% of the potassium salt of 7-ADCA, are obtained in crystalline form.

Example 4: Purification of vinyl-ACA *via* the N-benzyl-tert.butylammonium salt of vinyl-ACA

4a) N-benzyl-tert.butylammonium salt of vinyl-ACA

5.13 g of vinyl-ACA, containing 1.0% of 7-ADCA, are suspended in a mixture of 22.6 ml of acetone and 6 ml of water. 5 ml of N-benzyl-tert.butylamine are added in one portion. A solution is obtained for a short time and crystallisation occurs. The crystal suspension is left to stand for 15 minutes, the crystal mass is agitated, and 200 ml of acetone are added dropwise within ca. 30 minutes. The suspension is stirred for a further ca. 105 minutes whilst cooling with ice, the precipitate is isolated, washed with acetone, and dried. 3.2 g of the N-benzyl-tert.butylammonium salt of vinyl-ACA are obtained in crystalline form.

Analysis

Vinyl-ACA	51.3 %	(HPLC)
7-ADCA	0.2 %	(HPLC)
N-benzyl-tert.-butylamine	40.4 %	(GC)
Acetone	7.0 %	(GC)
H_2O	2.0 %	(KF)

^{1}HNMR (D_2O, trimethylsilylpropionic acid sodium salt-d_4): 6.70 (dd, J=11 and 18, C=CH); 5.37 (d, J=18, C=CH_2); 5.20 (d, J=11, C=CH_2); 5.02 (d, J=5, H_7); 4.69 (d, J=5, H_6); 3.67 (d, J=17, H_2); 3.53 (d, J=17, H_2); 7.6 (m, ArH); 4.19 (s, CH_2); 1.42 (s, CH_3).

4b) Vinyl-ACA via the N-benzyl-tert.butylammonium salt of vinyl-ACA

2 g of the N-benzyl-tert.butylammonium salt of vinyl-ACA, obtained as described in Example 4a), are dissolved in 30 ml of water, and the solution stirred for 10 minutes at room temperature with 0.2 g of activated charcoal. The charcoal is filtered off and the filter washed with 10 ml of water. The filtrate is cooled to 5°. The pH is adjusted to 3.4 with 10 N H_2SO_4 within ca. 15 minutes. The suspension is stirred for a further 2 hours whilst cooling with ice, the precipitate is isolated, washed water and acetone, and dried. 1.03 g of vinyl-ACA, containing 0.3 % (HPLC) of 7-ADCA, are obtained.

Example 5: Purification of vinyl-ACA *via* the 2-ethyl-1-hexylammonium salt of vinyl-ACA

5a) 2-Ethyl-1-hexylammonium salt of vinyl-ACA

5.13 g of vinyl-ACA, containing 1.0% 7-ADCA, are suspended in a mixture of 22.6 ml of acetone and 4 ml of water, and 4.7 ml of 2-ethyl-1-hexylamine are added in one portion. A solution is obtained within ca. 5 minutes. 200 ml of acetone are added dropwise whilst stirring. The resultant suspension is slowly stirred for 17 hours at -10°. The precipitate is isolated, washed with acetone which has been cooled to -10°, and dried.

4.4 g of 2-ethyl-1-hexylammonium salt of vinyl-ACA are obtained in crystalline form.

Analysis:

Vinyl-ACA	55.9 % (HPLC)
7-ADCA	0.3 % (HPLC)
2-Ethyl-1-hexylamine	31.8 % (GC)

Acetone	11.1 % (GC)
H_2O	0.6 % (KF)

^{1}H-NMR (D_2O, trimethylsilylpropionic acid sodium salt-d_4): 6.72 (dd, J=11 and 18, C=CH); 5.39 (d, J=18, C=CH_2); 5.22 (d, J=11, C=CH_2); 5.05 (d, J=5, H_7); 4.72 (d, J=5, H_6); 3.70 (d, J=17, H_2); 3.56 (d, J=17, H_2); 2.91 (d, J=6, CH_2N); 1.60 - 1.70 (m, CH); 1.20-1.40 (m, CH_2); 0.85 (2xt, J=7, CH_3).

5b) Vinyl-ACA *via* 2-ethyl-1-hexylammonium salt of vinyl-ACA

2.5 g of the 2-ethyl-1-hexylammonium salt of vinyl-ACA, obtained as described in Example 5a), are dissolved in 40 ml of water and stirred for 10 minutes at room temperature with 0.25 g of activated charcoal. The charcoal is filtered off and the filter washed with 10 ml of water. The filtrate is cooled to 5°. The pH is adjusted to 3.4 with 10 N H_2SO_4 within ca. 10 minutes. The resultant suspension is stirred for 2 hours whilst cooling with ice. The precipitate is isolated, washed with water and acetone, and dried.
1.41 g of vinyl-ACA, containing 0.4% of 7-ADCA, are obtained.

Example 6: Purification of vinyl-ACA *via* chromatography

10 g of vinyl-ACA, containing 1.0% 7-ADCA, are dissolved in 40 ml of water by adding aqueous ammonia to a pH of 8.5. The solution is placed in a column filled with 300 ml of HP-20 resin. Elution with water is carried out, and the eluate is collected in 25 ml fractions. The fractions with <1.0 HPLC area% of 7-ADCA are combined and vinyl-ACA is precipitated by adding conc. hydrochloric acid to pH 3.5. The precipitate is filtered off, washed with water and acetone, and dried. The resin is purified by washing with 80% methanol, and conditioned again with water.
6.2 g of vinyl-ACA, containing 0.1% (HPLC) of 7-ADCA, are obtained.

Example 7: Purification of vinyl-ACA *via* chromatography

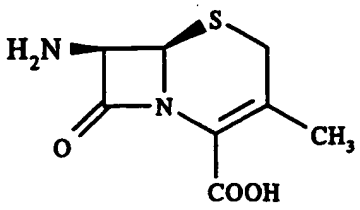
10 g of vinyl-ACA, containing 1.0% of 7-ADCA, obtained in fractions according to example 6, are dissolved by adding aqueous ammonia to a pH of 8.5. The solution is placed in a column filled with 350 ml of XAD-1600 resin and eluted with water. At a HPLC partition ratio of vinyl-ACA / 7-ADCA of 1:1, the first fraction is taken out and discarded. At a 7-ADCA content in the eluate of ca. 1.0 HPLC area%, the second fraction

is separated and used in a subsequent trial for dissolving the vinyl-ACA/7-ADCA starting mixture. The last fraction is acidified to a pH of 3.5 with conc. hydrochloric acid. The crystals obtained are filtered off, washed with water and acetone, and dried. The resin is purified by washing with 80 % methanol and conditioned again with water.

7.6 g of vinyl-ACA, containing 0.2 % (HPLC) 7-ADCA, are obtained.

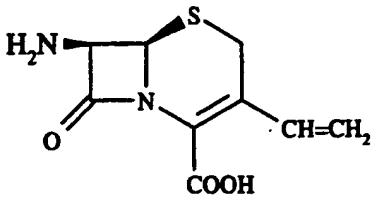
Patent Claims:

1. A process for the depletion of 7-ADCA of formula



II

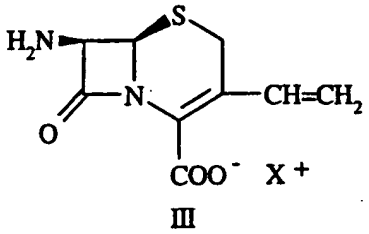
in a mixture of 7-ADCA and vinyl-ACA of formula



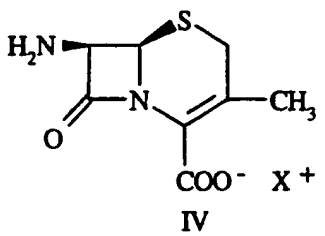
I

2. A process according to claim 1, wherein
   a) a mixture of a salt of a compound of formula I and a compound of formula II is subjected to crystallization, the crystallised salt is isolated and converted into a compound of formula I, containing less compound of formula II than the mixture of a compound of formula I and formula II, or
   b) a mixture of a compound of formula I and a compound of formula II is subjected to chromatography.

3. A process according to claim 2, wherein a mixture of a salt of a compound of formula I and a compound of formula II is a mixture of compounds of formulae

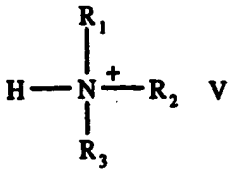


III



IV

wherein X^+ denotes a cation, or a compound of formula



V

wherein

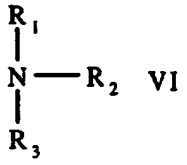
R_1, R_2 and R_3 are the same or different and independently of one another denote hydrogen, alkyl, aryl, aralkyl, or cycloalkyl; or

R_1 and R_2 together with the nitrogen atom form a heterocycle and R_3 is as defined above.

4. A process according to claim 2, wherein a mixture of a salt of a compound of formula I and a compound of formula II is produced by addition of a salt forming agent to a mixture of a compound of formula I as defined in claim 1 and a compound of formula II as defined in claim 1 in a solvent.

5. A process according to claim 4, wherein the salt forming agent is an inorganic base, an inorganic salt, an organic salt or a nitrogen base.

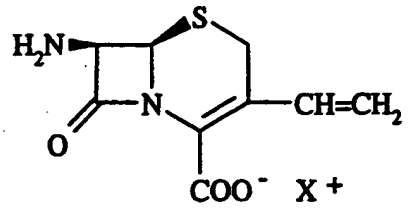
6. A process according to claim 5, wherein the inorganic base is a hydroxide; the inorganic salt is an inorganic alkali salt; the organic salt is an alkali salt of a carboxylic acid; and the nitrogen base is a compound of formula



VI

wherein R_1, R_2 and R_3 are as defined in claim 3.

7. A compound of formula



III

wherein X^+ is as defined in claim 3, in crystalline form.

8. Dicyclohexylammonium salt of 7-amino-3-vinyl-cephalosporanic acid in crystalline form

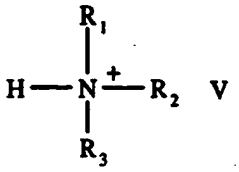
Tert.octylammonium salt of 7-amino-3-vinyl-cephalosporanic acid in crystalline form

N-Benzyl-tert.butylammonium salt of 7-amino-3-vinyl-cephalosporanic acid in crystalline form

2-Ethyl-1-hexylammonium salt of 7-amino-3-vinyl-cephalosporanic acid in crystalline form

Potassium salt of 7-amino-3-vinyl-cephalosporanic acid in crystalline form.

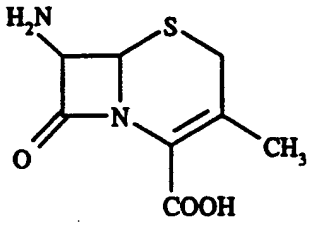
9. Use of a compound of formula



V

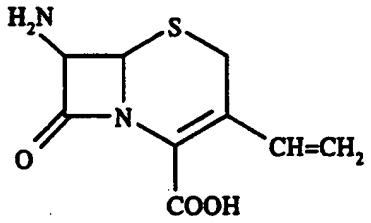
wherein X^+ is as defined in claim 3, or the use of a process as defined in claim 1, in the production of highly active cephalosporins.

10. Depletion process of 7-ADCA of formula



II

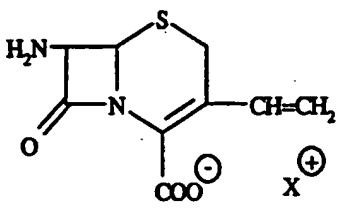
in mixtures of vinyl-ACA of formula



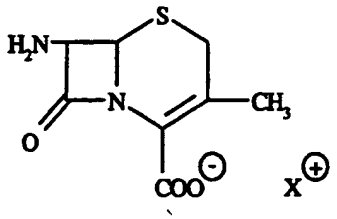
I

with 7-ADCA, characterised in that

a) a mixture of vinyl-ACA and 7-ADCA is converted into salts of formula

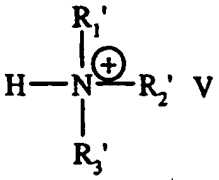


III



IV

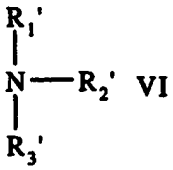
wherein $X^{\oplus}$ is $Li^{\oplus}$, $Na^{\oplus}$, $K^{\oplus}$ or a cation of formula



V

wherein R_1', R_2' and R_3' are the same or different and independently of one another denote hydrogen, (C_{1-8})alkyl, optionally substituted benzyl or phenyl or (C_{4-8})cycloalkyl, or R_1' and R_2' together with the nitrogen form a 5- or 6-membered heterocycle which optionally contains a further one or two hetero atoms, and R_3' is as defined above,

by reaction of the mixture of vinyl-ACA and 7-ADCA with a lithium, sodium or potassium base or with an amine of formula



VI

wherein R_1', R_2' and R_3' are as defined above, whereby

α) the reaction is carried out in a solvent or solvent mixture in which the salts of formulae III and IV have different solubilities, or

β) the salts of the compounds of formulae III and IV are suspended in a solvent or solvent mixture, and the solubility product is adjusted,

and after isolating the compound of formula III, this is converted using an acid into the compound of formula I having no content or a reduced content of 7-ADCA, or

b) a solution of a mixture of vinyl-ACA with 7-ADCA is subjected to chromatography.

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/EP 96/03582

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D501/18 C07D501/22 C07D501/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,93 16084 (BIOCHEMIE GESELLSCHAFT M. B. H.) 19 August 1993 see page 20 - page 23; claims	1-10
A	EP,A,0 503 453 (BIOCHEMIE GESELLSCHAFT M.B. H.) 16 September 1992 cited in the application see page 1	1
A	EP,A,0 597 429 (BIOCHEMIE GESELLSCHAFT M.B. H.) 18 May 1994 cited in the application see page 1	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 November 1996	28.11.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Luyten, H

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

information on patent family members

Intern al Application No
PCT/EP 96/03582

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9316084	19-08-93	AT-B-	399876	25-08-95
		AT-A-	19192	15-12-94
		EP-A-	0630380	28-12-94
		JP-T-	7503474	13-04-95
EP-A-503453	16-09-92	AT-B-	396106	25-06-93
		AT-B-	396238	26-07-93
		JP-A-	4327590	17-11-92
		US-A-	5401841	28-03-95
EP-A-597429	18-05-94	AT-B-	400436	27-12-95
		AT-A-	221292	15-05-95
		JP-A-	6247973	06-09-94